

The mutant Phe to Ala

Project N. _____
Block No. _____

23

Tag N. _____

① The same phenyl alanine corresponding to Tag polymerase will be changed to tyrosine

② For exo D will be changed to Alanine (corresponding region of Tag).

Brian cloned the SphI fragment of the Pol into M13mp.

I isolated the single stranded DNA from CJ236 as described before in Bio rad manual.

Test 5µl ssDNA

The DNA looks real good.

For D-A (3'-5' exo mutant oligo) is

5' GA | CGT | TTC | AAG | CGC | TAG | GGC | AAA | AGA # 2899
EcoRII site

For Phe → Tyr (O-helix)

~~GA~~ GTA | TAT | TAT | AGA | GTA | GTT | AAC | CAT | CTT | TCC | A
HpaI site
2904

Brian kinased 2899 before.

kinased 2904 as follows:

- 2µl oligo (210 pmol)
- 6µl 5X buffer (350mM Tris pH7.6, 50mM MgCl₂, 50mM KCl, 5mM PMS)
- 1µl 10mM ATP
- 0.5µl T4 Kinase (5U)
- 20.5µl H₂O
- 5' at 37°C → Heat at 65°C + 3 min TE

Read & Understood by me,

[Signature]

Date

4/8/95

Invented by

Recorded by

[Signature]

Date

3/7/95

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Oligof 2899
d 2900

REQUEST FOR SYNTHESIS OF OLIGONUCLEOTIDES

Date: 2/21/95
Name: BRIAN J. SCHMIDT
Department: GENETIC ENGINEERING
Account: 0530

Cost: \$ 168

Micromole Scale: PR

1'-75' exo mutant T-nuc REVERSE oligo Create Eco 47III (SDN1)

GA / CGT / TTC / AAG / CGC / TAG / GGC / AAA / AGA ^{3'}

5'-73' exo mutant T-nuc (SDN1) (AntII site).

^{5'} AAC / GCC / GTC / TAT / GAC / GTC / GCC / AGG / ATG / CTC ^{3'}

Brief Description Of Use:

(I) #2899 ✓
(II) #2900 ✓

APPROVAL:

D. Hattge
DIRECTOR OR VICE PRESIDENT

2.22.95 FL
DATE RECEIVED

2.24.95 FL
DATE SYNTHESIS COMPLETED